Workshop on the Genetic Transformation of Cowpea

Capri, Italy
October 31 – November 2, 2002

The organizers gratefully acknowledge the contributions made to this workshop by the following organization:

The Rockefeller Foundation

Acknowledgements

The achievement of this meeting owes much to Dr. Stephania Grillo, who made the local arrangements, and to Katy Ibrahim, who handled the day-to-day logistics and the predictable difficulties with her customary cheerfulness. Sincere thanks as well to Professor Luigi Monti and to Dr. Edgardo Filippone for their support and encouragement.

Executive Summary

Cowpea, an orphan crop of Africa grown predominantly by women, has substantial unrealized promise as a food and as a cash crop. The supply of cowpea is limited by numerous abiotic and biotic factors, but insects, which attack the crop both in the field and in storage after harvest, are the most serious problem. Despite a quarter-century of effort by IITA, the Bean/Cowpea CRSP, USA scientists as well as National Programs researchers in various African countries, breeding for genetic improvement to create insect-resistant cowpeas has been stalled by the lack of useful resistance genes in the cowpea germplasm pool. At the same time, for more than a decade it has been clear that novel insect resistance genes, such as those encoding Bt Cry proteins or α-amylase inhibitor, could in principle be used to introduce needed resistance to key insect pests into cowpea if it were possible to genetically transform cowpea. Early efforts to transform cowpea were hindered by the difficulty of transforming this legume, the small size of the scientific community involved, the lack of interest by industry, as well as by insufficient and unsustained funding.

Fifteen international scientists concerned with the genetic improvement of cowpea recently convened at a workshop organized by NGICA (Network for the Genetic Improvement of Cowpea for Africa) at Villa Orlandi, Capri, Italy on October 31-November 1, 2002. The Rockefeller Foundation provided financial support for the meeting, with supplementary funding from the Bean/Cowpea CRSP as well as other sources. Purposes of the workshop were to: (1) assess the state-of-the-art regarding the genetic transformation of cowpea; (2) report on progress of ongoing research and share information, experiences, and ideas; (3) develop mechanisms for improved communications among the various research groups involved; (4) review genes available to impart insect resistance through genetic transformation; (5) agree upon plans to verify and exploit breakthroughs, and; (6) seek mechanisms to address the many associated intellectual property issues.

The meeting brought together scientists with actively-funded projects for genetic transformation of cowpea, as well as other participants who have experience with the transformation of large-seeded legumes. Each group shared details of its ongoing work, and its future plans. Lines of communication between laboratories were opened, plans were made for exchanging materials or for mutual visits, and ideas about the best tactics or approaches were shared. Several researchers not currently working on cowpea genetic transformation indicated that they will seek financial resources to enable them to become engaged in the effort. Emerging from the Capri discussions was a sense of community among the researchers, and an agreement to quickly share any advances. This will make possible rapid verification of any breakthrough in transformation as well as hasten its dissemination and further development in various laboratories. Alternatives for dealing with intellectual property matters were examined. At the end of the meeting the scientists agreed upon a series of considerations and resolutions which reflect the values and approaches that will be honored as the work proceeds.

NGICA Considerations and Resolutions

Fifteen scientists interested in the genetic improvement of cowpea convened at a workshop organized by NGICA (Network for the Genetic Improvement of Cowpea for Africa) at Villa Orlandi, Capri, Italy on October 31-November 1, 2002. The Rockefeller Foundation provided much of the financial support for the meeting. Purposes of the workshop were to: (1) assess the state-of-the-art regarding the genetic transformation of cowpea; (2) report on progress of ongoing research and share information, experiences, and ideas; (3) develop mechanisms for improved communications among the various research groups involved; (4) review genes available to impart insect resistance through genetic transformation; (5) agree upon plans to verify and exploit breakthroughs; and (6) seek mechanisms to address the many associated intellectual property issues. The assembled scientists adopted the following considerations and resolutions:

1. This group acknowledges that cowpea (*Vigna unguiculata*), a grain legume, is a key food for some two hundred million people in sub-Saharan Africa. It is also a source of cash income for tens of millions of low resource farmers, many of them women. Cowpea fixes nitrogen and thrives in dry environments where few other crops can, but it suffers from numerous constraints. Its productivity is far lower than its potential.

2. The goal of the NGICA network, which includes numerous African cowpea breeders, is to develop technological improvements of cowpea that can reach farmers in the form of genetically-superior cowpea seeds.

3. Many important problems of cowpea agriculture have proved to be intractable or difficult using traditional plant improvement regimes. Biotechnology approaches should be used wherever and whenever they are the only viable solution, or if they are likely to provide a more favorable benefit-to-cost ratio than other approaches.

4. Because the genetic transformation of cowpea, together with allied applications of biotechnology (marker-assisted selection tools, genetic maps, and BAC libraries) holds the promise of increased food availability for the benefit of the people of sub-Saharan Africa, appropriate aspects of biotechnology should be explored and developed to the point where their adoption can be realized.

5. Decisions regarding the acceptability, adoption, safety and deployment of biotechnology-derived genetic improvements of cowpea in African countries rest rightfully, properly, and solely in the hands of the peoples and nations of Africa.

6. Roles of NGICA scientists include providing scientific and technological assistance in a collaborative and/or advisory mode, minimizing any bias and taking into account not only potential benefits but also any probable risks, with the longer-term goal of fostering capacity-building within African institutions.

7. To speed the introduction of needed genetic improvements into cowpea, all potential public and private contributors are urged to recognize that multiple, parallel initiatives to relieve a series of constraints (environmental safety, food safety, intellectual property, seed production and dissemination, policy, resistance management, public information) require timely and reliable support if they are to converge into a viable, practical and useful technology.
8. NGICA's efforts to introduce novel genetic traits into cowpea will respect intellectual property laws and meet proper biosafety requirements. This will require experienced legal and regulatory counsel and the resources to obtain it.

9. The genetic modification of cowpea is an extremely complex undertaking that will involve contributions of many institutions and individuals. To prevent duplication in research, address gaps, and spur on the process, a mechanism is needed to coordinate and stimulate all necessary activities. NGICA is prepared to continue to assist in the process.

10. Given the magnitude and complexity of genetic improvement of cowpea and the substantial resources required, the participation of additional contributors, public and private is welcomed. Help is especially sought from those research organizations which already have substantial experience with genetic transformation of large-seeded legumes.

Participating Scientists

Richard Allison, Dept. of Plant Biology, Michigan State University
Illimar Altosaar, Department of Biochemistry, University of Ottawa
George Bruening, University of California-Davis
Ray A. Bressan, Department of Horticulture, Purdue University
Deborah Delmer, Office of Food Security, The Rockefeller Foundation
Frederick Erbisch, Consultant on Intellectual Property Matters
Edgardo Filippone, University of Naples
T.J. Higgins, Plant Industry, CSIRO
Joseph E. Huesing, Consulting entomologist
Ivan Ingelbrecht, International Institute of Tropical Agriculture
Hans-Joerg Jacobson, University of Hanover
Luigi Monti, Professor, University of Naples
Larry L. Murdock, Department of Entomology, Purdue University
Idah Sithole-Niang, Department of Biochemistry, University of Zimbabwe
Nancy Terryn, IPBO, University of Ghent

Proceedings Notes

Prof. Luigi Monti, University of Naples, Chair. Professor Monti welcomed the participants and gave a brief overview of earlier cowpea transformation work carried out in conjunction with the Italian-led Joint Cowpea Biotechnology Programme. He pointed out that several of the present participants had enjoyed a long and cordial working relationship on cowpea going back to the late 1980's. The aim was to now expand that group and ensure good coordination of efforts.

Larry Murdock, Purdue University, Opening Remarks and Introductions. Prof. Murdock acknowledged the leadership role played by Prof. Monti in cowpea research beginning in the 1980's. He recalled the historic Italian/IITA cowpea collaboration, which focused especially in the areas of germplasm acquisition and characterization, and biotechnology, and mentioned the involvement of Purdue scientists. The goal of the present meeting is to understand the state of the art of cowpea transformation (and legumes generally). Several of the participants are new to the NGICA group and were warmly welcomed and invited to participate. The bottom line is that there is not now an efficient, robust, reproducible method of cowpea transformation, and we need to help create one.

Deborah Delmer, the Rockefeller Foundation. Dr. Delmer gave an overview of the Rockefeller Foundation (RF) and its program on African crops improvement and the role of biotechnology. This included an overview of the African Agriculture Technology Foundation (AATF) and how it might relate to the purpose of the NGICA. She also communicated the lessons learned from the rice biotechnology program. Cowpea and common bean are two of about 8 crops of interest to RF given that RF efforts for rice are winding down and focus is shifting to Africa. In Africa, there are three themes: 1) Improve crop varieties (Joe DeVries), 2) Enhance soil productivity (John Lynam), and 3) Increase markets and incomes for farmers (Akin Adesina). As regards improved crop varieties (Joe DeVries and John O'Toole) these include nutritional improvement, resistance to drought, viruses, fungal and bacterial diseases, insects, and striga. Other concerns they address relate to providing global public goods for poor farmers, such as creation of new enabling technologies, IP management, and public/private partnerships.

The ultimate RF goal is to put improved crops into the hands of farmers. This requires an integrated approach, including training, infrastructure, molecular approaches, conventional breeding, and seed delivery systems. Of additional interest is the RF venture capital group ProVenEx (http://www.rockfound.org) that has invested in seed companies in Kenya and Uganda.

Dr. Delmer shared some of RF experiences with rice. She pointed out that it is important that: 1) people must know and trust each other, 2) each participant has a defined task(s), 3) people occasionally work with collaborators, 4) there are meetings to share results, 5) all participants assume ownership overall and 6) they share information.

In terms of the appropriate use of GM-crops from the RF perspective, the following insights were shared. The use of GM-crops will be explored when conventional breeding cannot yield a solution, *e.g.,* the trait is not in the germplasm, the goal is altered nutritional quality, or for specialty products such as vaccines. GM-crops will also be supported when the benefits of the technology outweigh the costs and when IP and regulatory issues can be addressed.

Currently, there are three major issues from RF's perspective: 1) public perception, 2) freedom to operate (FTO) and 3) regulatory. Specifically addressing the FTO issue are two new initiatives at RF. The first is Intellectual Property sharing within the public sector headed by Debbie Delmer. The second is public-private partnerships like the AATF. The advice being given in the public sector is to avoid exclusivity licenses for specialty crops. The RF is also developing databases and management tools and technology in the public domain.

Of special note to NGICA is the AATF. Their mandate is to work with the private sector to facilitate transfer of proprietary research tools to be used for sub-Saharan Africa only. The need is there since companies need assurances for stewardship and liability of donated or purchased technology. In essence, the AATF will act as a kind of firewall. Eugene Terry is currently the Implementing Director. AATF will be African-based and have African leadership. Some of the conditions for AATF support are that it be scientifically sound, there be an unmet need, and that stewardship be addressed. The AATF does not fund the project but can help broker the funding. They also act as a clearinghouse. A new Website now exists for the AATF (http://www2.merid.org/AATF/).

Larry Murdock, Purdue University: Cowpea Overview -- Constraints and Promises.
Prof. Murdock reviewed the uses, area in production, trade, biotic and abiotic constraints, processing prospects, seed sources, and avenues to genetic improvement. The ideal cowpea plant was described: it would: (1) have bruchid and *Maruca* (legume pod borer) resistance, (2) multiple disease and virus as well as striga resistance, (3) produce large white seeds, (4) 70-80 day maturity, (5) show good leaf retention, (6) present its pods above the canopy, (7) be semi-determinate (good lateral branching), and preferably adapted to the growing areas where *Maruca* is most serious. Eventually, the ideal plant would include thrips as well as pod-sucking bug resistance. It would be best if the genotype transformed could have many of the above traits to begin with, but whatever the genotype first transformed, backcrossing should enable the Bt or other genes to be moved into acceptable agronomic types fairly rapidly and efficiently. The drought tolerance work of Tony Hall at Riverside and B.B. Singh at IITA was mentioned. Several slides illustrating the nutritional, economic, supply/demand and constraint aspects of cowpea were shown -- work currently being led by Jess Lowenberg-DeBoer and Augustine Langyintuo at Purdue. Biotic pests of cowpea include: striga, *Maruca*, pod-sucking bugs, cowpea bruchid, thrips, viruses, nematodes and bacterial diseases. The magnitude of the insect pressure on cowpea is evident with insecticide sprays, which in some cases and circumstances can increase yields as much as 20-fold.

Idah Sithole-Niang, University of Zimbabwe-Harare: NGICA -- Overview and Perspective. Dr. Sithole-Niang addressed the mission and nature of NGICA. She stated its broad goal as developing genetic technologies to address cowpea constraints that will ultimately lead to improved production. Farmers can get improved technology in the form of better seeds with built-in genetic improvements. Emphasis of NGICA right now is on molecular tools but they are a smaller part of the entire NGICA strategy which also encompasses, for example, conventional breeding. Indeed, plant breeders are of absolutely central importance to cowpea improvement, both with regards to traditional breeding/screening as well as biotechnology. She also addressed membership of NGICA, and shared the resolutions from the 2001 Dakar, Senegal meeting. She pointed out that the NGICA newsletters have been well received. NGICA activities to date include (1) developing links with industry, (2) encouraging economic studies of cowpea in Africa, (3) obtaining funding for (i) environmental safety research and for (ii) management of insect resistance genes, as well as (4) working out a comprehensive NGICA flow chart , and (5) organizing the meeting of researchers interested in cowpea transformation. Dr. Sithole-Niang pointed out that the transformation technology would probably be developed in a single country first and then moved to others as feasible. She also said that there are no funds currently allocated for NGICA management activities and we need, minimally, some funding for web-management and administration. Dr. Sithole - Niang also discussed Maarten Chrispeel's useful booklet on Foods from Genetically Improved Crops in Africa. Some small NGICA funds were used to help get the booklet translated into French, to serve the Francophone region of Africa.

Prof. Luigi Monti, University of Naples -- the Joint Cowpea Biotechnology Programme -- History, Perspective, Progress, and Lessons Learned. The Joint Cowpea Biotechnology Programme funded by the Italian government operated from 1988 to 1995. In the original program, priority was given to insect resistance. Many institutions were involved in Italy, the USA, UK and Africa, particularly IITA. Prof. Monti pointed out that the program there had been successful with cowpea transformation but that the bottleneck was the very low regeneration rate of plants. One of the major successes of the program was the establishment of the Biotechnology laboratory at IITA. Based on the support of the meeting participants, Prof. Monti indicated his interest in taking a proposal forward to the Ministry of Foreign Affairs to continue the cowpea work.

A question arose related to biosafety issues, specifically, "Are there biosafety guidelines for Nigeria." Dr. Ivan Ingelbrecht answered that there are guidelines, but there is no government agency to implement them. A National Biosafety Committee needs to be established under the auspices of the Federal Ministry of the Environment. The goal is to bring in one or two transgenic crops into Nigeria. In Kenya and South Africa, there is still more work needed as well.

Hans-Joerg Jacobsen -- Grain Legume transformation: where are we now? Prof. Jacobsen has worked on grain legume transformation since the late 1980s. The crops include *Pisum, Cicer, Vicia, Lens,* and *Phaseolus* but not yet cowpea. He is in the process of preparing a review paper on legume transformation. In general, legumes are

very difficult to transform. The major breakthrough was by T.J. Higgins and colleagues using an *Agrobacterium*-mediated approach. This is the approach that Prof. Jacobsen likes as well. Using *in vitro* grafting techniques he can get T1 plants in about 11 months. The approach uses embryonic tissue plus *Agrobacterium* followed by antibiotic treatment to remove the *Agrobacterium*, selection for herbicide resistance (bar-gene) and finally *in vitro* grafting. With *Pisum* he has a transformation efficiency of 0.2 to 0.3% and with *Vicia* about 0.3%. This equates to 50 clones with 5 different antifungal genes, for example, in an affordable amount of time. With *Pisum*, 60 transformation experiments yielded about 20 explants within 2 years, or 0.3% (60 transgenic lines from 20,000 explants). He had two observations to share. First, the first shoots from decapitated embryos are never transgenic but are loaded with Agro. He thinks the active meristems are resistant to *Agro* transformation. He must add cytokinin to induce shoot formation; the newly induced shoots quite often are transgenic. Second, auxin seems to stimulate transformation. He gave several examples of this approach used with pea, *Faba*, and chickpea. He pointed out that this procedure did not work with *Phaseolus*. In this crop one apparently needs a callus phase before regeneration, but the calli die after the transformation. Apparently there are problems associated with polyphenol production under selection. The non-selected cells are killed, producing polyphenols, which in turn kill the few transformed cells. Likewise, the gene gun has not been successful except apparently at EMBRAPA. Debbie Delmer asked whether MARS help, and T.J. Higgins' response was that they do not.

Fred Erbisch: Guidance on Intellectual Property Issues. Dr. Erbisch spoke to the importance of IP protection for the project. Liability is a big issue. He suggested a NGICA clearing group for IP management. It was not clear however, as to how we might implement that. Clearly, IP is something the group needs to address but a working group model is not in place as yet. See more below as this issue was discussed at length later in the meeting. Dr. Erbisch also distributed IP accounting sheets.

Ray Bressan, Purdue University -- Drought tolerance genes and other prospects for African Crops; Bean/Cowpea CRSP-supported cowpea transformation work at Purdue. Prof. Bressan states that it is vital to have an efficient morphogenesis/transformation system. The key point, which he reiterated several times, is the skill of the person working on the transformation system. He also emphasized that one needs an appropriate vector with high infection efficiency and a good selectable marker (*e.g.*, the bar gene). Also, one should choose the best cowpea genotype. He points out that *Agro* must have access to the germ. Also discussed was the Baby Boom gene, which is a conserved gene in a number of plants and which affects meristem development. Illimar Altosaar pointed out that the IP surrounding Baby Boom is a big issue.

Prof. Bressan next gave an overview of salt and drought tolerance work. Using a "Harvest Index" he pointed out that specific genes can have a big effect on root and leaf morphology and growth, cell growth and division, flowering time *etc*. The idea is to ID the genes that control the aforementioned traits because you want fast growth when water is available. A summary point is that drought tolerant plants grow with minimal

resources but grow really fast when resources are available. He also emphasized that the aforementioned genes are highly conserved in many species. He speculates that site-directed gene introduction would be used in the future.

Richard Allison/Idah Sithole-Niang, Michigan State U. and U. Zimbabwe/Harare -- Rockefeller-sponsored work toward electrical transformation of cowpea. Prof. Allison described an electrophoresis system under development for introduction of DNA into cowpea. Their model system uses the cowpea aphid-borne mosaic virus (potyvirus) capsid protein gene, driven by 35S and containing a NPTII selectable marker and *Gus* reporter gene. The transformation system uses whole plants and electrophoresis into the apical meristem. Nine variables, such as seedling maturity, were tested. Of 6,000 seedlings, 5 were found to be positive by Gus assay. At the time of the meeting there was no molecular characterization. Prof. Allison also has used Bar and found that 4 plants were resistant to Liberty herbicide and positive by Southern analysis as well. It was suggested that he use a PCR-based approach instead of Southern blots. Dr. Nancy Terryn agreed to advise Dr. Allison on the use of PCR for this application. As an aside, there was considerable discussion as to whether this approach had been used before by others. Dr. Edgardo Filippone offered a reference, Ahokas *et al*., 1989, Theor. Appl. Get 77:469-472, where the approach may have been previously described. In any event, Prof. Alison has filed a patent disclosure describing the technology.

George Bruening, UC Davis -- USAID-sponsored cowpea transformation work at UC Davis. Prof. Bruening discussed work underway in his laboratory to transform cowpea. Prof. Bruening is looking at nine variables and systematically testing for their effects. His approach involves cotyledenary node generation. A reference given was: Anand, R.P. 2001. Current Science (Bangalore) 80:671-674. Prof. Jacobsen suggested he stop somatic embryogenesis work, stating that auxin for 10 minutes is enough to induce embryogenesis. Prof. Bruening is also involved with BAC library construction (5-7X coverage) for genetic mapping for Striga resistance genes.

Ivan Ingelbrecht -- The cowpea transformation program at IITA, Ibadan, Nigeria. Dr. Ingelbrecht described the cowpea transformation program at IITA, which has been underway since the early 1990s. He acknowledged that his presentation was built upon work done by Paul Keese during 2002 and before that time by Jesse Machuka. Dr. Ingelbrecht gave a brief introduction on the various procedures that exist for genetic transformation of plants. He mentioned that the procedure now followed at IITA was focused on the use of cotyledonary nodes as explants. Experiments were conducted using two cowpea genotypes that were most responsive to multiple shoot formation. Kill curves have been determined for kanamycin, hygromycin and phosphinotricin and large-scale transformation experiments using hygromycin as selective agent were underway. Related research topics addressed by IITA are gene flow and the effect of Bt toxins on non-target organisms. IITA's work with wide-crosses and embryo rescue has been discontinued. Prof. Bressan made several points regarding the selection pressure needed for success. He said that the pressure needs to be very high. If plants die under high selection then you do not have transformation. In the past apparently people have used low selection since the total number of positives with high selection is so very low. Prof. Bressan's

point is that the positives one gets with too low selection pressure are false positives. Further, he believes that the tissue culture person is critical. You also need very healthy cultures. It is a number game in his opinion and the more shoots the better. Finally he suggests one should use Basta instead of hygromycin.

T.J. Higgins -- Rockefeller-supported cowpea transformation program at CSIRO. The goal of the project is to produce a reliable plant regeneration and gene transfer system. The approach Dr. Higgins is using is based on chickpea. Briefly, the seed is germinated overnight, and then split. The cotyledon is the target using the wounded shoot tip from the embryonic axis. Shoot induction medium is TDZ, which results in multiple abnormal shoots.

For regeneration, six methods were tested and the soybean method was found to be best (B5:BAP). They have tested 20 cowpea cultivars so far, with regeneration after 4 and 8 weeks. Selectable markers in use or contemplated are NptII, Bar (Aventis/Bayer) and Hpt. The uses of these are being discussed with the CSIRO's legal council because of IP issues. The construct being used is Bar (NptII) [35S]RB-LB[PHA]-AAI. Post-doctoral researcher Carlos Popelka is carrying out the work in Canberra.

Idah Sithole-Niang -- Bean/Cowpea CRSP-supported initiative to use RNAi to reduce flatulence factors in cowpea. Still in the early stages of planning, the idea is to use RNAi technologies to silence the genes encoding enzymes involved in specific sugar production associated with flatulence.

Larry Murdock -- Bioassay data, Bt and *Maruca*, alpha-amylase inhibitor and Cowpea weevil; a status report. Dr. Murdock presented an overview and data pertaining to testing of select *Bacillus thuringiensis* endotoxins against *Maruca* pod borer as well as testing of alpha amylase inhibitor (AAI) and avidin against cowpea weevil.

In terms of Bt activity against *Maruca* the following data were presented.

Bt Toxin	**LD50 Activity in ppm**	**LD90 in ppm**
Cry1Ac	0.43	5.60
Cry1Ab	0.03	0.09
Cty1C	0.08	0.40
Cry2Aa	0.09	0.56
Cry1Aa	1.00	2.20

All of the *Cry* proteins tested were isolated and purified from individual *E. coli* strains transformed with a single *Cry* gene. They were provided by Prof. Bill Moar at Auburn University. In terms of AAI, published data are available demonstrating the activity against cowpea weevil (Shade et al., 1994, Bio/Technology 12: 793-796). Good unpublished data are also available for avidin. Avidin is from chicken egg white where it functions as a biotin-binding protein. It is stable to human gut protease but is not heat stable. Genes encoding several of these proteins are good candidates for imparting insect resistance to cowpea.

Illimar Altosaar -- Available Bt's for cowpea transformation, IP matters, and experiences and lessons learned in collaborative international projects. Prof. Illimar Altosaar gave an overview of work in his laboratory covering insect-resistant transgenic rice as well as IGF and virus vaccine work. He works with rice, carrot and tobacco. Illimar started out working on promoters. At the urging of Rockefeller he became involved in the development of Bt-rice for insect control. In 1993, he obtained the constructs for Cry1Ab and Cry1Ac. A paper describing the work was published in April 1997 (Cheng, PNAS 95:2767) and (Nester et al., 2002 – ASM Colloquium). http://www.asm.org. The goal is multi-toxin plants to be used for insect resistance management. Ideally, each toxin should contain a different binding site. Prof. Altosaar's Bt-rice (KMDI) is in its 15th generation. A rice actin promoter drives the gene. The transformation plan for multi-toxin Bt-rice is to produce individual single gene lines that will then be crossed to attain the stacked genes. The construct described is pRD400_D35S_AMV_1Ac/1C/2A_Nos-ter_pRD400. Prof. Altosaar pointed out that rice farmers are rich relative to African farmers. He also mentioned work to produce a Bt coffee tree with activity against leaf miner.

Currently, Prof. Altosaar has MTA's with 25 countries for 28 crops. Forty labs have MTA's for rice. His major criterion is stewardship in terms of negotiating with the labs for signing. Genes in Illimar's collection are Cry1Ab (Monsanto), 1Ac (Monsanto), 1C (65% GC) from Don Dean, 1C (45% GC) from Vitality Company, and 2A from L. Masson. Vitality was a company in Israel but is now out of business. Illimar invited participants who wish to use these genes to contact him.

Edgardo Filippone, University of Naples -- Cowpea and chickpea regeneration and genetic transformation work at University of Naples. Dr. Filippone has been working on cowpea transformation for ten years. In terms of regeneration work, approaches used include callus morphogenesis, which leads to multiple bud cluster proliferation. Dr. Filippone suggests revisiting the somatogenesis issue again. Several explants have been tested (*e.g.,* leaf, cotyledon, nodes, etc.) as well as different hormone levels (auxin/cytokinin). For example, chickpea (desi type form India and Kabuli type from Europe) were used in conjunction with work with T.J. Higgins.

In terms of genetic transformation work, Dr. Filippone has used direct methods such as electro-diffusion, which did not work, electroporation, and particle bombardment. A measure of his systems efficiency was given. A point stressed by Dr. Filippone was the need to transform the germ line (L2-L3) and not L1. He suggests you need a lot of shoots at various stages to get a few with transformed L2-L3 lines. Dr. Filippone has also used *Agrobacterium* using Gus and endochitinase (chickpea). The project on cowpea was ended when funding from IITA dried up.

Nancy Terryn, IPBO, Ghent -- *Phaseolus* transformation work at Ghent and CIAT -- applications to cowpea? Dr. Terryn is affiliated with Prof. Marc Van Montagu at the Institute Plant Biotechnology for Developing Countries (http://www.ipbo.rug.ac.be). A useful site to visit is: http://www.phaseolus.net. An overview of the group's work was given along with work they have done in collaboration with CIAT, Columbia. They have

worked with *Phaseolus acutifolius* (Tepary bean) (see Dillen *et al*., Theor. Appl. Genet. 94: 151-158, 1997, De Clercq et al, Plant Cell Reports 21: 333-340, 2002).

Their work with *Agrobacterium* does not result in chimeras, as many meristem based protocols do. Approaches they have used involve using TDZ as the cytokinin in the medium, co-cultivate at 22 degrees and gradually increase levels of geneticin (5 to 20 mg/ml). They use carbenicilin starting at 500 mg/ml to kill *Agrobacterium*. Multiple shoots are easy to get with the use of coconut water. Roots however are difficult to obtain so if needed they graft onto WT seedlings. With this approach they never get chimeras and have a 1:3 segregation ratio in the progeny. They score for Gus after one hour. They are using arcelins (lectin-like storage proteins for which they have a 3D structure) and are thought to be involved in *Zabrote*s resistance, as well as other genes of interest (De Jaeger et al, 2002). Starting with 50 beans they get 5 to 10 transformants. This approach works for *P. acutifolius* but not *P. vulgaris*. They are also looking at genes that up-regulate the cell cycle (RepA and CycD) as well as genes involved in embryogenesis (Lec2, Wuschel, and Baby Boom) to improve transformation rates

Issues -- Discussion led by Larry Murdock, Idah Sithole-Niang and Joe Huesing. Two major areas were discussed in follow-up. The first was Intellectual Property. Dr. Fred Erbisch strongly advised that we formulate a mechanism to cover IP issues for NGICA. Dr. Erbish suggested that we form an NGICA repository for IP issues. While a good concept, it was not clear at present how we would do that since each investigator is responsible to his/her institution on matters of IP. However, Dr. Erbisch's suggestion does highlight both the need and the difficulty of managing the IP aspects of the program. Prof. Jacobsen agreed to forward the German/European guidelines on IP to Drs. Erbisch and Huesing after the meeting. The formation of AATF might go a long way to settling this issue for NGICA, if cowpea was taken by AATF.

The second area discussed at length was the considerations and resolutions document. All were satisfied that these captured the essence of the meeting and accurately addressed the next steps in the cowpea transformation process. As part of that discussion Prof. Luigi Monti was encouraged to seek funding from the Italian government to revisit cowpea transformation. Also Dr. Nancy Terryn will seek funding from the Belgian authorities.

Cowpea Genetic Transformation Workshop
Capri, Italy Oct. 31-Nov 2, 2002
Tentative Program

October 31, Thursday

8:30 AM	Prof. Luigi Monti, University of Naples, Chair. Welcome
8:40 AM	Opening Remarks and Introductions -- Larry Murdock
9:00 AM	Deborah Delmer, the Rockefeller Foundation: [the Rockefeller Foundation, its program on African crops improvement, role of biotechnology, lessons learned from the rice biotechnology program, overview of the AATF and how it might relate to NGICA]
9:40 AM	Larry Murdock, Purdue University: Cowpea, an Overview -- Constraints and Promises [cowpea in Africa: uses, area in production, trade, biotic and abiotic constraints, processing prospects, and seed sources, avenues to genetic improvement]
10: 20 AM	Coffee break
10:40 AM	Idah Sithole-Niang, University of Zimbabwe-Harare: NGICA -- Overview and Perspective
11:10 AM	Prof. Luigi Monti, University of Naples -- the Joint Cowpea Biotechnology Programme -- History, Perspective, Progress, and Lessons Learned
11:40 AM	Hans-Joerg Jacobsen -- Grain Legume transformation: where are we now?
12:15 PM	Fred Erbisch: Guidance on Intellectual Property Issues
12:30 PM	Lunch

2:30 PM	Ray Bressan, Purdue University -- Drought tolerance genes and other prospects for African Crops; Bean/Cowpea CRSP-supported cowpea transformation work at Purdue
3:10 PM	Richard Allison/Idah Sithole-Niang, Michigan State U. and U. Zimbabwe/Harare -- Rockefeller-sponsored work toward electrical transformation of cowpea
3:50 PM	George Bruening, UC Davis -- USAID-sponsored cowpea transformation work at UC Davis
4:30 PM	Discussions
8:00 PM	Group dinner

November 1, Friday

8:30 AM	Ivan Ingelbrecht -- The cowpea transformation program at IITA, Ibadan, Nigeria
9:10 AM	TJ Higgins -- the Rockefeller-supported cowpea transformation program at CSIRO
9:50 AM	Idah Sithole-Niang -- Bean/Cowpea CRSP-supported initiative to use RNAi to reduce flatulence factors in cowpea
10:30 AM	Coffee break
11:00 AM	Larry Murdock -- Bioassay data, Bt and *Maruca*, alpha-amylase inhibitor and Cowpea weevil; a status report
11:20 AM	Illimar Altosaar -- Available Bt's for cowpea transformation, IP matters, and experiences and lessons learned in collaborative international projects.
12:00 PM	Edgardo Filippone, University of Naples -- Cowpea and chickpea regeneration and genetic transformation work at University of Naples
12:30 PM	Lunch
2:30 PM	Nancy Terryn, IPBO, Ghent -- *Phaseolus* transformation work at Ghent and CIAT -- applications to cowpea?

3:10 PM Issues -- Discussion led by Larry Murdock, Idah Sithole-Niang and Joe Huesing

1. How can we best foster the image (and the reality) that will best advance the cause?
2. Intellectual property management -- how do we organize to handle it?
3. When the transformation breakthrough occurs -- what then?
4. Genotypes? What would be best for the breeder, farmer and consumer?
5. Site-specific promoters?
6. How do we work together? -- Communications among ourselves?
7. The gene flow problem with cowpea.
8. Resistance management in a new environment.
9. Other issues arising.

November 2, Saturday

7:30 AM Discussions (cont). NGICA Strategy session -- what, beyond transformation, ought we be giving more attention to? Resolutions.

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